In another embodiment of test kit, only a fraction of the analyte-specific receptor is capable of binding to the immobilized ligand. Such a kit may comprise (i) immobilized in or on a membrane a ligand which binds specifically to the receptor, and (ii) dissolvably pre-deposited in or on the membrane a specified amount of analyte-specific receptor substance, only a specified fraction of which is capable of binding to the immobilized ligand.

Still another embodiment of test kit may comprise (i) dissolvably pre-deposited in or on a membrane a first specified amount of analyte-specific receptor substance, and (ii) immobilized in or on the membrane a second specified amount of the analyte-specific receptor substance.

In an alternative embodiment, the solid phase is a solid phase well, such as a microtiter plate well. Such of test kit may comprise a solid support having one or more wells with the second amount of analyte binding receptor immobilized therein and with the first amount of analyte-binding receptor dissolvably pre-deposited in the well or in close contact with the well.

In the following, the invention will be illustrated in more detail by a specific non-limiting Example.

EXAMPLE 1

Immunoassay for C-reactive protein (CRP) in undiluted serum samples Measuring range 10 – 200 mg/l

Principle

Sample is mixed with biotinylated anti-CRP-fab in excess and the mixture is applied to a test strip having a deficient amount of streptavidin in the reaction zone. After an intermediate wash, anti-CRP fluorophore-conjugate is added and after a wash, conjugate that has bound to the reaction zone is measured. Since only a small part of the biotinylated anti-CRP-fab can bind to the reaction zone the consumption of the fluorophore conjugate is reduced considerably.

Test strips

5 x 48 mm nitrocellulose membranes (Whatman, porosity 8 µm) on a polyester backing were used. The strips had a sample application zone at one end and a

downstream reaction zone with immobilized streptavidin in an amount capable of binding approximately 6% of biotinylated anti-CRP added in the assay procedure.

Samples

CRP-containing samples of varying CRP concentration were prepared from a 200 mg/l of recombinant CRP (Fitzgerald) in hCRP depleted serum.

Procedure

15 µl of biotinylated anti-CRP-fab (monovalent fab-fragment of monoclonal antibody) and 15 µl of CRP-containing serum were mixed and the mixture was applied to the application zone of the membrane strip. The amount of biotinylated anti-CRP-fab was 3 µg per test strip, which is a 2 x molar excess of anti-CRP in relation to the standard 200 mg/l CRP. After an intermediate wash with 15 µl of test buffer (50 mM borate buffer pH 8.0, 3% BSA, 5% sucrose, 0.15 M NaCl, 0.005% $CaCl_2$, 0.05% NaN_3), 15 µl of detection conjugate solution [3 µg of anti-CRP monoclonal antibody (Fitzgerald) coupled to 0.1 µm TransFluoSpheres-SO_4/CHO (633/720 nm) (Molecular Probes Inc.), the above test buffer] were added , followed by wash with 2 x 15 µl of test buffer. The fluorescence of the strip was then measured. The results are shown in Table 1 below.

Table 1

CRP conc. (mg/l)	Peak area obtained (V x mm)
0	0.08
0	0.07
10	2.56
10	2.50
30	3.62
30	4.01
100	5.24
100	4.87
200	6.28
200	5.82

EXAMPLE 2 (comparative)

Immunoassay for CRP in serum samples diluted 1/20

Measurement range 10 – 200 mg/ml

Principle

Sample is diluted in test buffer and applied to test strips having an excess of anti-CRP in the reaction zone. Anti-CRP fluorophore-conjugate is then added followed by a wash, whereupon conjugate that has bound to the reaction zone is measured. Sample dilution is necessary to avoid unreasonably large amounts of anti-CRP in the reaction zone as well as in the detection conjugate.

Test strips

5 x 48 mm nitrocellulose membranes (Whatman, porosity 8 µm) on a polyester backing were used. The strips had a sample application zone at one end and a downstream reaction zone with 2.6 µg immobilized anti-CRP monoclonal antibody (Fitzgerald), which is a 13 x molar excess in relation to a standard 10 mg/ml CRP serum.